OBSERVATIONS ON THE USE OF THE THERMAL ENERGY ANALYZER AS A SPECIFIC DETECTOR FOR NITROSAMINES

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The use of the Thermal Energy Analyzer (TEA) is becoming more widespread for the analysis of nitrosamines (NAs). Originally the TEA was available as a gas-liquid chromatography (GLC) detector (Fine & Rounbehler, 1975a), but it has recently been interfaced with a high-performance liquid chromatograph (HPLC) (Fine et al., 1976a). Based on TEA detection and mass spectrometric (MS) confirmation, NAs have been found in air (Fine et al., 1976b,c), water (Fine et al., 1976d), cutting oils (Fan et al., 1977), pesticides (Fan et al., 1976), and corrosion-inhibiting formulations (Archer & Wishnok, 1976). These findings have stimulated considerable interest, since they suggest that the presence of nitrite, either alone or produced from nitrate, with a nitrosatable amine will produce NAs in the environment, even under mild conditions.

There is no question that the TEA is more highly specific for NAs than GLC detectors, such as thermionic or alkali flame ionization, Coulson and electron-capture, since it measures the liberated nitric oxide radical. We are concerned about reports based on NA data obtained by TEA alone without mass spectral confirmation, particularly since Fine et al. (1975b) have determined that a number of non-NAs or impurities in the sample give a TEA response. These include organic nitrites, C-nitro and C-nitroso compounds. In our own work on the determination of volatile NAs in cured meats, we have noted a number of GLC-TEA peaks that elute prior to nitrosodimethylamine (NDMA), in confirmation of the report by Stephany & Schuller (1976). In addition, we have encountered samples giving peaks that eluted after NDMA, but which did not correspond exactly to the retention time of any of fourteen, known, volatile NAs.

We have developed a sensitive photolytic procedure that distinguishes between NAs and non-NAs that may serve as an aid to NA identification. This resulted from the ability to detect very low levels of apparent NAs by the TEA without the corresponding ability to confirm them by MS. We are reporting herein on naturally-occurring compounds that give a 'false' TEA response and the new photolysis procedure.

EXPERIMENTAL

Sample materials were either extracted directly with methylene chloride or digested by the multidetection procedure of Fazio et al. (1971). Nitrosoethylmethylamine (NEMA) and sometimes nitrosohexamethylenimine (NHMI) were used as internal standards.

Nitrosamine determinations were performed on a GLC interfaced with a TEA operated under conditions similar to those described by Fine et al. (1975b) except when the cold trap temperatures were varied. A 280 cm x 3 mm o.d. column packed with 15% Carbowax 20M-TPA on 60-80 mesh Gas Chrom P was programmed from 130 to 210° at 4°/min with an argon carrier flow rate of 84 ml/min. The relative response, r, of the non-NA was determined by dividing its peak area per mole by the peak area per mole of NDMA, using one-half width times the height. The r of diethylnitramine was calculated similarly but was compared to nitrosodiethylamine.

Twenty microliters of methylene chloride solutions of standard NAs, sample extracts, or concentrates, were introduced into thin-walled, melting-point, capillary tubes (1.6-1.8 x 100 mm), placed ca. 5 cm from a 366 nm ultraviolet lamp and exposed for 2 hr. Unphotolyzed control samples were obtained by covering the capillary tubes with opaque material and treating them in an identical manner. Both photolyzed and unphotolyzed samples were run by GLC-TEA. Additional details of this procedure are described elsewhere (Doerr & Fiddler, 1977).

The identities of the compounds under investigation were established by comparing the GLC-TEA retention times with those of authentic compounds and by combined GLC-MS, operated in the peak matching mode at a resolution of 1 in 12,000 under conditions previously described (Pensabene et al., 1974). Nitrosamines are potentially carcinogenic and should be handled with care.

RESULTS AND DISCUSSION

The normal operating conditions of the TEA recommended by the manufacturer involves the use of a liquid nitrogen-isopentane slurry (-150°C) for the cold trap. This trap is designed to remove the sample solvent and pyrolysis fragmentation products while allowing the nitric oxide radical to pass into the reaction chamber. A GLC-TEA chromatogram of a methylene chloride extract of tobacco smoke condensate, run at the recommended cold trap temperature (-150°C), is shown in Figure 1 (curve a). The two peaks at 3.8 and 13.7 min corresponded

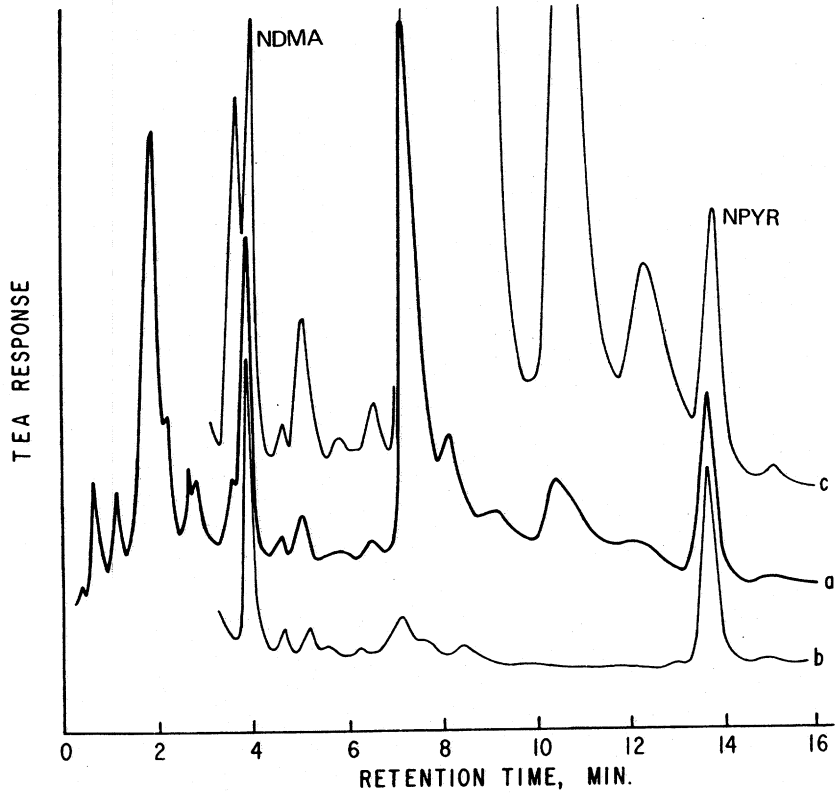
to the retention times of authentic NDMA and nitrosopyrrolidine (NPYR), respectively, and were subsequently identified as these compounds by high-resolution mass spectrometry. The larger peak at 7.3 min eluted between nitrosoethylpropylamine and nitrosodipropylamine, as shown by using a standard mixture of fourteen NAs. A chromatogram of the same sample, using a dry ice-acetone (-80°C) cold trap, is shown in Figure 1 (curve b), where the large peak at 7.3 min has disappeared completely, while the other peaks after NDMA remain the same. With the use of an ice-water (0°C) cold trap (Fig. 1, curve c) the two NA peaks are unchanged, but the peak at 7.3 min is very large. Comparing the chromatograms using the three trap temperatures, there is also a marked difference in the number and size of the peaks eluting prior to NDMA. Virtually identical chromatograms were obtained when the cold trap was maintained at room temperature and at 0°. Except for a slightly smaller NPYR peak, the chromatograms were identical when liquid nitrogen (-196°C) was used in the cold trap, instead of the liquid nitrogen-isopentane slurry. The peak at 7.3 min was identified as pyrrole by low and high resolution ms. The relative response, r, of the purified compound was determined to be 0.0015. With such a weak TEA response, the quantities of pyrrole in the concentrate had to be very large in order to produce such a significant peak. Of major interest is the fact that an authentic sample of pyrrole gave no TEA response when a dry ice-acetone cold trap was used, but did give a weak response at lower temperatures. *N*-methylpyrrole (r = 0.0067) behaved in a similar fashion, but 2,5-dimethylpyrrole (r = 0.059), a compound also giving a weak TEA response, did not. It gave a diminishing but positive response with decreasing trap temperature. The reason for this behaviour is not known at present. These results suggested that a -80°C cold trap would be most desirable for NA analyses; however, such was not the case. Concentrates of a number of different sample types, particularly imported cheese, chromatographed with the trap at -80°C, contained peaks in the NDMA (3.8 min) to nitrosomorpholine (15.0 min) region that were not present with the liquid nitrogen-isopentane trap. The quantification of NDMA is especially difficult because of the presence of interferring peaks. We now use liquid nitrogen almost exclusively in the cold trap, since it eliminates the preparation and maintenance of the liquid nitrogen-isopentane slurry.

Since most NAs undergo photolytic decomposition when exposed to u.v. light, this property was utilized in the development of an analytical method, sensitive to pg concentrations of the NAs. Initially, the procedure was applied to check the validity of the TEA response to NAs. The chromatogram of unphotolyzed, tobacco-smoke, condensate extract with nitrosoethylmethylamine (NEMA) added as an internal standard is shown in Figure 2, curve a, and one of the same sample after photolysis in Figure 2, curve b. The latter shows that the three NA peaks have disappeared completely, while pyrrole was unaffected.

This same photolytic procedure combined with GLC-TEA detection has been applied to methylene chloride concentrates derived from fried

FIG. 1. GLC-TEA CHROMATOGRAMS OF TOBACCO SMOKE CONDENSATE EXTRACT FOR DIFFERENT TEA COLD TRAP TEMPERATURES

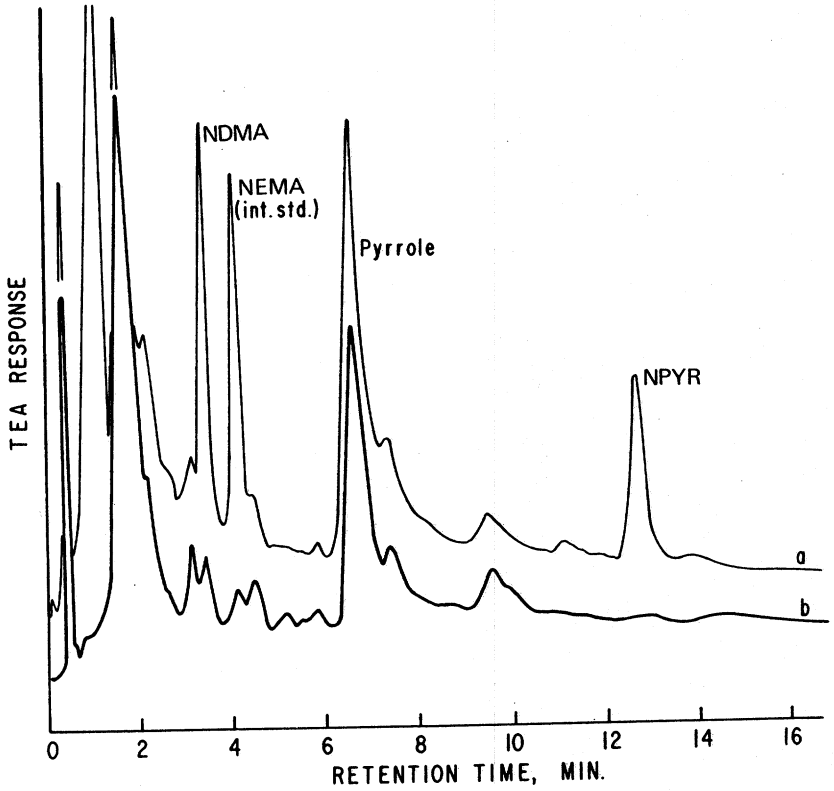
a. liquid nitrogen-isopentane; b. dry ice-acetone; c. ice-water



bacon and, to a lesser extent, to cheese and fish products. In addition, it has been most useful in providing further evidence for the presence of volatile NAs in deionized water (Fiddler et al., 1977), gastric contents (Lakritz et al.[1]) and saliva, where the levels

1 See p. 425

FIG. 2. GLC-TEA CHROMATOGRAM OF TOBACCO SMOKE CONDENSATE EXTRACT
a. unphotolyzed; b. photolyzed



found are too low, or the samples too dirty, to establish their identity by MS. Some interesting observations were made during these investigations. For example, a Tilset cheese sample appeared to contain 4 ppb NDMA and 14 ppb NPYR, neither of which could be confirmed by MS. Photolysis and re-chromatography indicated that the 'NPYR' peak consisted of only 5 ppb NPYR; the remainder of the peak consisted of unidentified non-NA(s). In addition to possible difficulties in placing quantitative values on apparent NAs, a limited number of smoked salmon samples gave an indication of the presence of volatile NAs other than NDMA and of non-NAs, shown by the fact the peaks were

unaffected by photolysis. One of these peaks had the same retention time as diethylnitramine which has a relative molar response of 0.35 compared with the corresponding NA. The identity of a number of non-photolyzable TEA peaks is currently under investigation. One of the possible difficulties in using photolysis is the formation of secondary reaction products. None was observed except in fish samples, but even these did not limit the usefulness of the procedure. While our experience with photolysis of food products containing volatile NAs has been good, this technique is not intended to replace, but to supplement, MS confirmation. Caution should be exercised in calling TEA peaks NAs *without* having MS confirmation since there is considerable evidence to suggest that the TEA is not as specific as originally claimed.

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Reference to brand or firm names does not constitute endorsement of products by the U.S. Department of Agriculture over others of a similar nature not mentioned.

REFERENCES

Archer, M.C. & Wishnok, J.S. (1976) Nitrosamine formation in corrosion-inhibiting compositions containing nitrite salts of secondary amines. *J. Environ. Sci. Health, A11*, 583-590

Doerr, R.C. & Fiddler, W. (1977) Photolysis of volatile nitrosamines at the picogram level as an aid to confirmation. *J. Chromatogr., 140*, 284-287

Fan, S., Fine, D.H., Ross, R., Rounbehler, D., Silvergleid, A. & Song, L. (1976) *Determination of N-nitroso pesticides in air, water and soil. J. Agr. Food Chem.* (in press)

Fan, T.Y., Morrison, J., Rounbehler, D.P., Ross, R., Fine, D.H., Miles, W. & Sen, N.P. (1977) *N*-nitrosodiethanolamine in synthetic cutting fluids a part per hundred impurity. *Science, 196*, 70-71

Fazio, T., Howard, J.W. & White, R. (1971) *Multi-detection method for analysis of volatile nitrosamines in foods.* In: Bogovski, P., Preussmann, R. & Walker, E.A., eds, N-*Nitroso Compounds - Analysis and Formation*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* No. 3), pp. 16-30

Fiddler, W., Pensabene, J.W., Doerr, R.C. & Dooley, C.J. (1977) The presence of dimethyl- and diethylnitrosamines in deionized water. *Fd Cosmet. Toxicol.* (in press)

Fine, D.H. & Rounbehler, D.P. (1975a) Trace analysis of volatile *N*-nitroso compounds by combined gas chromatography and Thermal Energy Analysis. *J. Chromatogr., 109*, 271-279

Fine, D.H., Rufeh, F., Lieb, D. & Rounbehler, D.P. (1975b) Description of the Thermal Energy Analyzer (TEA) for trace determination of volatile and non-volatile *N*-nitroso compounds. *Anal. Chem., 47*, 1188-1191

Fine, D.H., Hoffmann, F., Rounbehler, D.P. & Belcher, N.M. (1976a) *Analysis of* N-*nitroso compounds by combined high performance liquid chromatography and Thermal Energy Analysis.* In: Walker, E.A., Bogovski, P. & Griciute, L., eds, N-*Nitroso Compounds - Analysis and Formation*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* No. 14), pp. 43-50

Fine, D.H., Rounbehler, D.P., Belcher, N.M. & Epstein, S.S. (1976b) *N*-Nitroso compounds: detection in ambient air. *Science, 192*, 1328-1330

Fine, D.H., Rounbehler, D.P., Pellizzari, E.D., Bunch, J.E., Berkley, R.W., McCrae, J., Bursey, J.T., Sawicki, E., Krost, K. & DeMarrais, G.A. (1976c) *N*-Nitrosodimethylamine in air. *Bull. Environ. Contam. Toxicol., 15*, 739-746

Fine, D.H., Rounbehler, D.P., Belcher, N.M. & Epstein, S.S. (1976d) N-*nitroso compounds in air and water.* In: Walker, E.A., Bogovski, P. & Griciute, L., eds, N-*Nitroso Compounds - Analysis and Formation*, Lyon, International Agency for Research on Cancer (*IARC Scientific Publications* No. 14), pp. 401-408

Pensabene, J.W., Fiddler, W., Gates, R.A., Fagan, J.C. & Wasserman, A.E. (1974) Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. *J. Food Sci., 39*, 314-316

Stephany, R.W. & Schuller, P.L. (1976) *How specific and sensitive is the Thermal Energy Analyzer.* In: Tinbergen, B.J. & Krol, B., eds, *Proceedings of the Second International Symposium on Nitrite in Meat Products, Zeist, 1976*, Wageningen, Centre for Agricultural Publishing and Documentation, pp. 249-255